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Review Article

Genetic and environmental factors influencing human diseases with telomere dysfunction

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Abstract: Both genetic and environmental factors have been implicated in the mechanism underlying the pathogenesis of serious and fatal forms of human blood disorder (acquired aplastic anemia, AA) and lung disease (idiopathic pulmonary fibrosis, IPF). We and other researchers have recently shown that naturally occurring mutations in genes encoding the telomere maintenance complex (telomerase) may predispose patients to the development of AA or IPF. Epidemiological data have shown that environmental factors can also cause and/or exacerbate the pathogenesis of these diseases. The exact mechanisms that these germ-line mutations in telomere maintenance genes coupled with environmental insults lead to ineffective hematopoiesis in AA and lung scarring in IPF are not well understood, however. In this article, we provide a summary of evidence for environmental and genetic factors influencing the diseases. These studies provide important insights into the interplay between environmental and genetic factors leading to human diseases with telomere dysfunction.

Key words: Telomeres, telomerase, environmental factors, aplastic anemia, dyskeratosis congenita, idiopathic pulmonary fibrosis

Basic biology of telomere and telomerase

Telomeres are composed of simple repetitive DNA sequences [e.g., (TTAGGG)n in vertebrates], which are located at the ends of linear chromosomes [1]. Telomeric DNA consists of double-stranded region proximal to the centromere and the 3' distal single-stranded region (**Figure 1**). The single-stranded region has been shown by electron microscopic (EM) technique to be embedded in between the dsDNA region and is held in place by many telomere-binding protein factors in a unique DNA-protein macromolecular structure known as the T-loop (**Figure 1**) [2]. The proper formation of this specialized structure plays important biological roles, such as to protect chromosomes from illegitimate recombination, end-to-end fusion and degradation, and also to regulate telomere lengths *in cis*.

Telomerase enzymatic complex provides a way for the complete synthesis of the chromosome 3' ends. Replication of the ends of chromosomes poses a special problem for the conventional semiconservative replication machinery of the cells [3, 4]. As a result, telomeric DNA sequences located at the ends of chromosomes are progressively lost at each round of cell division [5, 6]. Normal mammalian somatic cells in culture can proliferate to a finite number of replication with the maximum number being referred to as the Hayflick limit [7], which can act as a molecular clock to monitor the replicative history of the cells [8]. A survey of over 90% of human cancer cells, which are immortal, reveals high levels of telomerase activity [9]. The expression of telomerase alone was found to be sufficient to immortalize a number of human cell types [10-12]. It is important to note, however, that ectopic expression of telomerase together with activation of oncogenes or with inactivation of tumor suppressor genes can sometime induce tumorigenic conversion of normal human cells [13]. These studies indicate that telomerase plays an important role not only in the normal cellular aging process but also in cancer development. Hence, understanding the

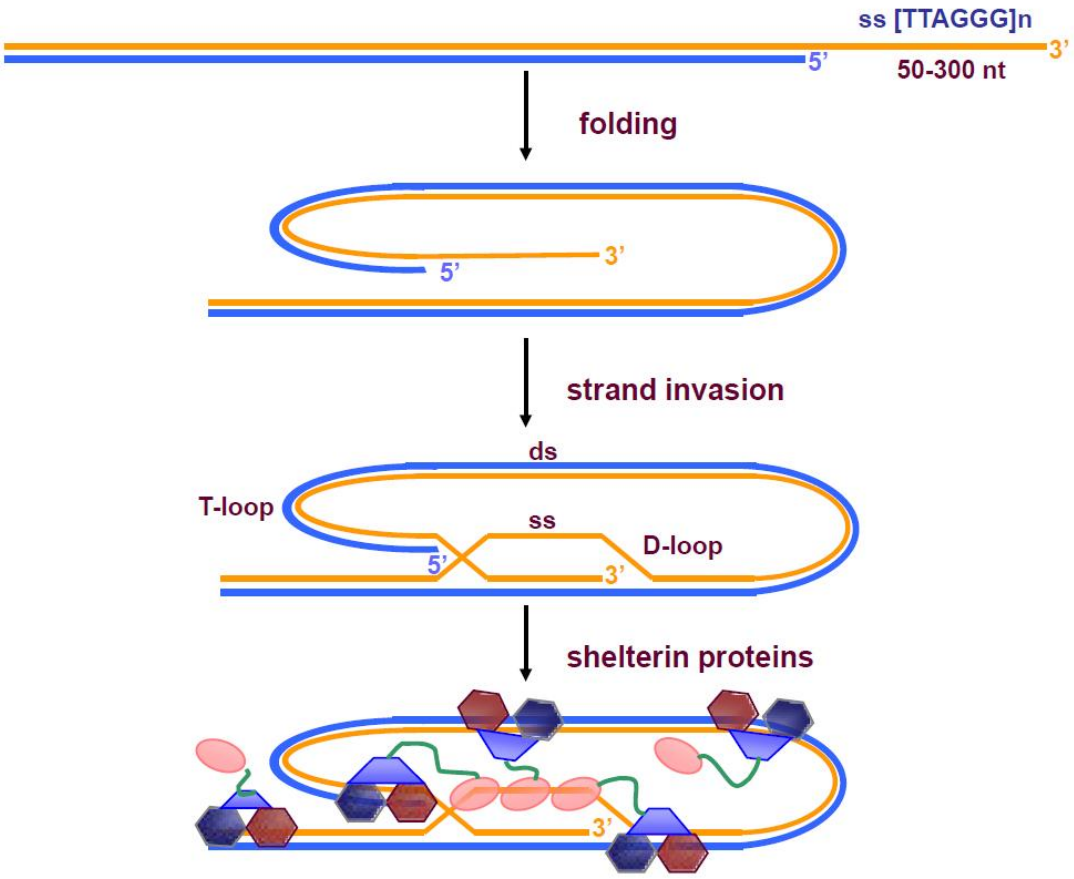

Figure 1: Linear chromosome end consists of the double stranded DNA sequence and the single-strand DNA (ssDNA) of repeated sequence (TTAGGG)n. The T-loop structure is formed by a strand-invasion event of the terminal 3' ssDNA into the double stranded telomeric sequence, and is composed of telomere associated with the telomere-binding protein factors of the shelterin complex.

structures and functions of telomeres and of telomerase that help maintain telomere lengths and chromosome stability in cells is of great importance to human health.

Telomerase is a ribonucleoprotein (RNP) complex with two main components: a protein (TERT) with RNA-dependent-DNA polymerase activity, and an integral RNA (TER or TERC) that provides a template to synthesize telomeric DNA repeats [14]. The ability to reconstitute human telomerase enzymatic activity *in vitro* using either synthetic hTER RNA and *in vitro*-transcribed and translated hTERT protein [15, 16] or ectopic expression of these two components in telomerase-negative human cells [12] has greatly advanced the field and suggests that hTER RNA and hTERT protein are the minimal functional components of the enzymatic complex. However, assembly of a functional telomerase holoenzyme complex also requires other telomere- and/or telomerase-associated proteins (e.g., dyskerin, NOP10, GAR1, NHP2,) (**Figure 2A**) [1, 17].

Telomerase catalytic proteins (TERT) from evolutionarily distant organisms share a conserved structural organization that can be divided into three functional domains (**Figure 2B**) [18]. At the N terminus are the telomerase-specific domains [19] that are required for functional assembly of the enzyme complex by mediating TERT interaction with its TER RNA partner and the homodimerization of the protein (i.e., TERT protein-protein interaction) [20, 21]. The functional reverse transcriptase (RT) domain

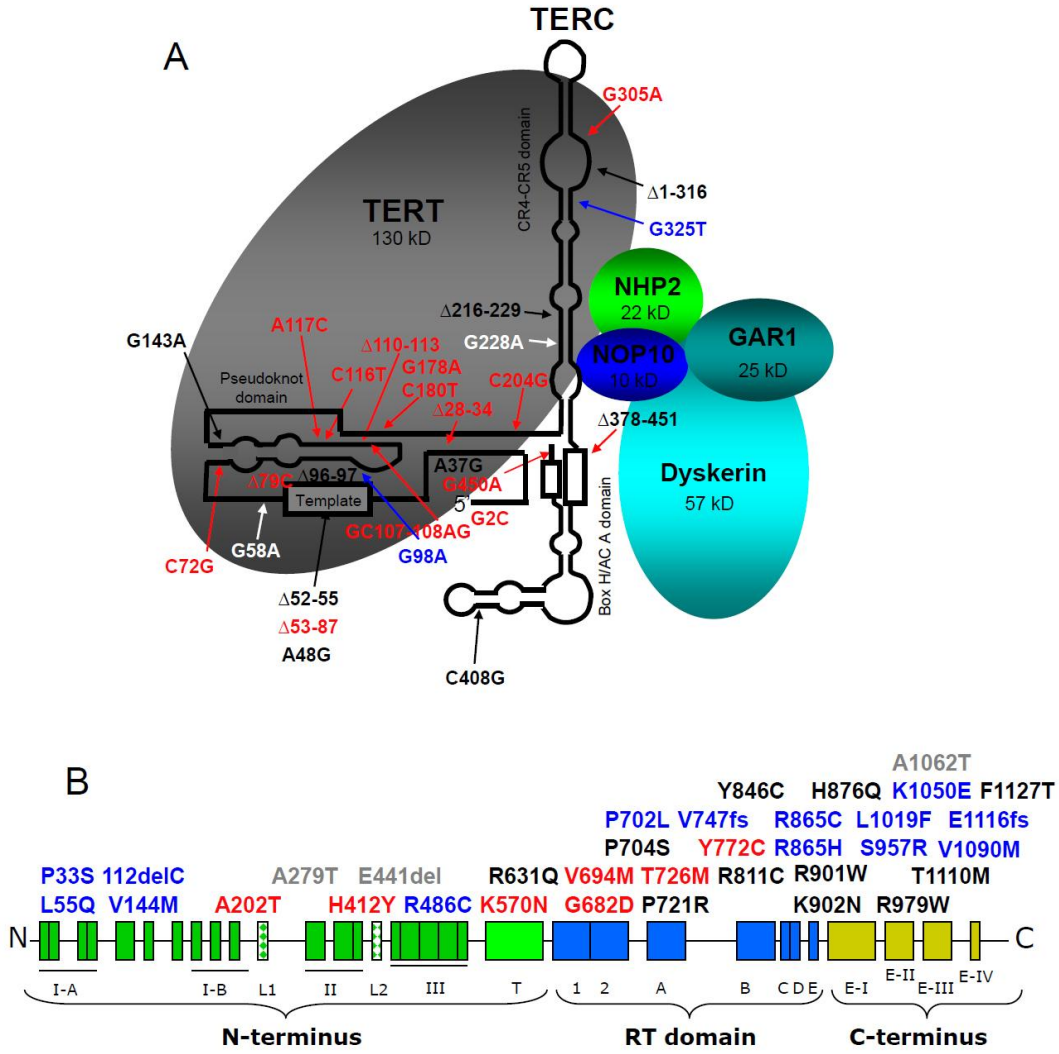

Figure 2: (A) Schematic diagram of the telomerase RNP complex. Template sequence of TER (or TERC) RNA (nts 46-53) and other conserved structural domains (CR4-CR5, pseudoknot, and Box H/ACA) are indicated. The representative DC-associated TER variants are shown in black, AA-associated mutations in red, and IPF mutations in blue. Rare SNPs (G58A and G228A) that have been found in both patients and healthy controls are shown in white. The TER-associated proteins Dyskerin, NHP2, NOP10 and GAR1 are also shown. (B) A linear depiction of human TERT protein with some of its known natural sequence variations is shown. IPF-associated mutations are shown in blue, DC-associated mutations in black, AA-associated mutations in red, and rare SNPs in gray. RT: reverse transcriptase domain.

with the universally conserved RT motifs is almost centrally located (**Figure 2B**) [14, 22]. The fact that mutations of key residues known to affect the conventional RT catalytic activity also negatively influence telomerase activity strongly argues that telomerase RT domain is the catalytic domain of the enzyme complex [22, 23]. The C-terminal domains of TERTs are also required for telomerase-specific enzymatic activity and/or in the telomeric nucleotide addition processivity process [14, 22].

Telomerase RNA template genes (*TER* or *TERC*) from numerous organisms have been cloned [24-27]. Mammalian TER RNAs are universally expressed in many tissues and throughout the development of the organisms,

even in tissues with no readily detectable telomerase activity [24, 28]. In contrast, the high level of telomerase catalytic protein expression is limited to cells with high replicative potential, suggesting that telomerase protein, instead of the TER RNA component, is the limiting factor in the formation of an active telomerase complex in cells. Despite the divergence of primary sequences and lengths among the >30 different vertebrate telomerase RNAs that have been analyzed, they all share a conserved secondary structure (**Figure 2A**), implicating an essential role of the properly folded structure of TER RNA in telomerase function [29]. The predicted human hTER RNA structure contains several conserved functional domains: a pseudoknot domain that contains the essential templating region and one of the binding sites of the catalytic hTERT protein, a conserved region CR4-CR5 that has been implicated as the secondary hTERT binding site, and an evolutionarily conserved box H/ACA and CR7 domain that have both been implicated to be important for hTER RNA accumulation, processing, assembly, and telomerase function in cells (**Figure 2A**) [30-35].

The box H/ACA motif of the human hTER has been found to be required for its association with four proteins (i.e., dyskerin, NHP2, NOP10, and GAR1) (**Figure 2A**) that are common in one of the two classes of the small nucleolar RNAs (snoRNAs) found in the nucleolus of the cells [31, 34]. Unlike those cellular snoRNAs, which function to modify other cellular RNAs, there is no evidence that hTER RNA functions in this capacity, however. Emerging evidence suggests that hTER RNA instead belongs to a new class of RNAs of the small Cajal body-specific RNA family (scaRNAs) [33]. Besides interacting with the four proteins of the snoRNA family, hTER has also been shown to associate with a number of other cellular proteins (i.e., hStau, L22, hnRNP C1/C1, La, TCAB1, and its integral hTERT) that are involved in hTER stability, maturation, accumulation, and assembly of the functional telomerase RNP complex [17, 36, 37].

Human diseases of telomere dysfunction

Recent studies conducted by our laboratory and others have shown that telomerase dysfunction and/or telomere shortening may be causative for two different forms of human diseases affecting the bone marrow (e.g., dyskeratosis congenita and aplastic anemia) and the lung (e.g., idiopathic pulmonary fibrosis) [38-56]. Evidences for telomere-telomerase dysfunction in these conditions are summarized below.

Dyskeratosis Congenita (DC)

Dyskeratosis congenita (DC), an inherited bone marrow failure syndrome, was first described in 1975 in patients with muco-cutaneous features of reticular pigmentation of skin, nail dystrophy and oral leucoplakia [57]. A variety of other abnormalities that are characteristic of early aging syndrome have also been associated with the disease, ranging from the less severe symptoms such as damaged teeth, hair loss and graying, and short statue, to a more severe nature such as testicular atrophy, pulmonary, neurological, skeletal, ophthalmic disorders, gastrointestinal hemorrhage and a predisposition to malignancy [58]. The clinical manifestations of DC imply dysfunction in the stem cells, which affect mainly the rapidly dividing tissues such as skin, oral mucosa and bone marrow. The median age of mortality is 16 years with bone marrow failure as a principal cause of death [58]. Occasionally, bone marrow failure may occur in some patients before any sign of muco-cutaneous abnormalities, and therefore can sometimes be diagnosed as idiopathic aplastic anemia [46]. The main course of treatment for severe bone marrow failure is allogeneic hematopoietic stem cell transplantation [59-61]. However, patients who undergo bone marrow transplantation experience a high incidence of transplant-related complications such as severe mucositis, sepsis, hepatic venooclussive disease, microangiopathic hemolytic anemia, and pulmonary fibrosis [62, 63]. Three inheritance patterns have been defined in DC: X-linked recessive, autosomal dominant, and autosomal recessive [58]. While it is not yet clear what causes the autosomal recessive cases of DC; *DKC1* gene has been linked to the X-linked cases; and *hTER*, *hTERT*, and *TINF2* [64, 65] genes have been associated with autosomal dominant forms of the disease.

The availability of a large number of DC families with only male patients has allowed the accurate linkage analysis and subsequent positional cloning of a single gene (*DKC1)* on the Xq28 chromosome, which has now been

implicated in all cases of the X-linked DC [66-68]. *DKC1* encodes a 58 kDa dyskerin protein, which, together with other proteins (e.g., NOP1, NHP2 and GAR1) and the small nucleolar RNA, forms the specialized small nucleolar ribonucleoprotein complex (snoRNP) that is responsible for an important step of ribosome biogenesis (i.e., the modification and processing of the large nascent ribosomal rRNA into mature 18S and 28S subunits) [69]. Dyskerin has been postulated to be an active pseudouridine synthase enzyme, based on its sequence homology to the well-characterized yeast Cbf5 and bacterial pseudouridylate synthase, which are known to catalyze the isomerization of uridine residues of the ribosomal RNA and other small nucleolar snoRNAs to pseudouridines [69]. However, peripheral lymphocytes collected from patients with X-linked DC showed no significant defect in rRNA processing or pseudouridylation but contained much shorter telomere lengths than did the age-matched controls [38, 70]. This may relate to the fact that the RNA transcript of the telomerase hTER RNA subunit in X-linked DC cells is unstable, constituting approximately five-fold less steady-state level in patients relative to maternal carriers [71].

Direct evidence to link the DC disease to telomere and/or telomerase dysfunctions came from the discovery that the *hTER* gene is mutated in some cases of the autosomal dominant form of DC (AD DC) [38]. Cloning of the first disease-associated *hTER* variant was facilitated by the identification of a large family with a naturally occurring deletion of 821 base pairs on one of the chromosomes 3q that effectively removes the 3' 74 nts of the box H/ACA motif [38]. The deleted form of hTER was not detectable in primary tissues collected from these patients, consistent with the idea that the box H/ACA motif is required for hTER accumulation and indicating that hTER haploinsufficiency may play an important role in the disease. We and other researchers have recently identified a number of additional *hTER* variants from DC patients [38, 42, 46]. Interestingly, most patients are heterozygous carriers for the germline mutation in the *hTER* gene. To the best of our knowledge, however, only one patient in our cohort is heterozygous for two different *hTER* sequence variants [42]. Like the X-linked DC cases, lymphocytes collected from AD DC patients exhibited shorter telomere lengths than did normal age-matched controls [38, 72].

Members of DC families in earlier generations, who carry pathogenic *hTER* sequence variants, are diagnosed later and generally have less severe illness than individuals from later generations, supporting the "disease anticipation" theory [58]. It is likely that both telomere length and the nature of telomerase mutation play an important role in disease development. While it is more difficult to directly test this theory in humans, both hypotheses have been addressed in mouse models. A mouse strain that lacks the RNA component of telomerase (*mTER-/-*) has been developed [73, 74]. These mice, which lack detectable telomerase activity, were (surprisingly) viable for six generations and showed a telomere-length attrition rate of 4.8(+/-2.4 kb) per generation. Successful reproduction up to 6 generations could be due to the fact that telomere lengths in laboratory strains of mice are inherently much longer than those of humans. Nevertheless, studies performed on cells collected from the later generations of the *mTER-/-* knockout mice (i.e., fourth and older) showed critically short telomeres with aneuploidy and other chromosomal abnormalities such as chromosome end-to-end fusions [73]. These late generations of mice also showed signs of premature aging in highly proliferative organs as well as increased cellular apoptosis, decreased wound healing ability, defective spermatogenesis, testicular atrophy, and hematopoietic defects, all of which resemble symptoms of DC in humans [74-76]. Mice that lack the catalytic component of telomerase (*mTERT-/-*) have also been produced [77, 78]. Again, these mice did not show abnormality in the earlier generations but exhibited telomere shortening effect in late generations. Recent studies have suggested that the levels of mTERT mRNA in heterozygous mice are one-third to one-half the levels expressed in wild-type mice, which is similar to the reductions in telomerase RNA observed in mTER heterozygote [79]. These findings indicate that even a moderate reduction in telomerase gene expression in mice due to heterozygosity could have a profound impact on telomere maintenance, consistent with the phenotype seen in some patients who are heterozygous for either of the telomerase gene components. These observations suggest that haploinsufficiency of either the *TER* or the *TERT* gene in humans or mice may undermine telomere maintenance and thus leads to disease.

Aplastic Anemia (AA)

Aplastic anemia (AA) was first described by Paul Ehrlich in 1888 and has been recognized as the paradigm of bone-marrow failure syndromes due to its simplistic pathological finding of an 'empty' bone marrow appearance [80, 81]. It has been estimated that the incidence of AA worldwide is 2-5 per million per year [58]. The 'empty' bone marrow, the hallmark of the disease, leads to dangerously low levels of production of all three different blood cell lineages (erythrocytes, granulocytes, and platelets). Anemia leads to fatigue, dyspnea, and cardiac symptoms; thrombocypenia to bruising and mucosal bleeding; and neutropenia to sharply increased susceptibility to infection. AA has always been strongly associated with exposure to chemicals and drugs in the environment (see below). While the causes for a majority of the cases of AA are enigmatic, a number of the cases can be explained by immune-mediated destruction of hematopoietic stem cells and progenitor stem cells that would normally give rise to peripheral blood cells [82]. The best evidence for this comes from the fact that most AA patients are responsive to immunosuppressive therapies [82]. The cytotoxic T lymphocytes to attack the marrow stem cells are those that express Th1 cytokines, especially gamma-interferon, which can trigger Fas-mediated apoptosis of hematopoietic cells [83, 84]. However, why T cells are activated in AA is unclear, and, despite the best efforts, no autoantigen has been identified that can trigger the marrow destructive effect. A peculiar and consistent finding in this disease is that telomeres of blood cells in AA patients are unusually significantly shorter than those of age-matched control cells [71]. There is also a strong correlation between telomere loss and disease status and duration: telomere lengths in patients who respond to the conventional immunosuppressive therapy are similar to those of normal controls, while they remain short in non-responders and untreated patients [41, 71]. We and other researchers have recently identified germline *hTER* and *hTERT* disease-associated mutations in some patients with acquired AA [41, 43-45, 52, 53, 55, 71] and have shown that some of these mutations can explain telomere attrition and short life span of cells in carriers. Most if not all of patients with telomerase mutations are non-responsive to the conventional immune-suppressive therapy. Instead, clinical observations have suggested that androgen therapy can improve blood counts in as many as 60% of patients [85], and steroid sex hormones (e.g., androgen, estrogen and progesterone) have been shown to stimulate telomerase gene expression in various human cell types [17], suggesting that patients with telomerase haploinsufficiency may benefit from this or related forms of therapy.

Idiopathic pulmonary fibrosis (IPF)

At about the same time that Paul Ehrlich described the fist case of aplastic anemia in a pregnant woman [80, 81], Sir William Osler reported the first case of idiopathic pulmonary fibrosis (in 1892) with a grim prognosis: "Death occurred about three months and a half after the onset of the acute disease and the lung was two thirds of the normal size, grayish in color, and hard as cartilage." [86]. Today, this progressive and fatal lung disease still afflicts more than 5 million patients worldwide with no effective treatment. Even with the application of modern medicine, the prognosis for IPF is still dismal, with a median survival of 3-5 yrs after initial diagnosis [87]. The disease is characterized by diffuse interstitial fibrosis with enigmatic pathogenesis. IPF is the most common form of a class of lung diseases known as idiopathic interstitial pneumonias (IIP). It has been speculated that unknown endogenous or environmental stimuli lead to aberrant lung-epithelial cell activation and remodeling [88]. Activated epithelial cells are known to release potent fibrogenic molecules and cytokines, such as TGF-b-1, which promotes fibroblasts transformation into myofibroblasts that can mediate the architectural disruption of the lung parenchyma. Wang et al. have recently implicated caveolin-1 as an endogenous inhibitor of IPF, which is consistent with the fact that overexpression of caveolin-1 suppresses TGF-b-1-induced production of extracellular matrix protein by lung fibroblasts [89].

Like AA cases, a subset of IPF patients also appears to show a familial mode of disease inheritance. Two separate research teams have recently implicated dysfunction in telomere and telomerase pathways as a possible molecular mechanism underlying the pathogenesis of IPF [47, 56]. Based on an earlier observation that four of the seven individuals in a single family who were carriers

of a DC-associated hTERT mutation were also diagnosed with IPF [48], the authors of this study performed a follow-up study to identify 8% of individuals in a cohort of 73 kindreds to carry heterozygous mutations in the *hTERT* or *hTER* gene [47]. Using an unbiased linkage analysis approach, a separate group scanned the whole genome of individuals in two large and unrelated families to find ~12% of their cohort of families to have heterozygous mutations in the same components of telomerase [56]. Like those with DC or AA, IPF patients in these studies showed telomere shortening effect over time, which conferred a dramatic increase in their susceptibility to the disease.

Environmental factors influencing human diseases

Benzene-induced hematotoxicity and blood cancers in humans

Aplastic anemia has historically been strongly associated with exposure to chemicals, drugs or other agents in the environment [90]. Cases of benzene-induced aplastic anemia were first reported in the early 1900s [91, 92]. A strong association of marrow failure with industrial exposure to benzene led to a successful campaign to improve safety in the workplace in the U.S. by substituting toluene or naphtha for benzene [93]. Benzene exposure, however, continues to occur worldwide to workers in the oil, shipping, automobile repair, shoe manufacture, and other industries and to the general public due to emissions from gasoline and combustion of hydrocarbons and tobacco [94, 95]. Benzene exposure has been shown to cause blood disorders, including AA, myelodysplastic syndrome (MDS), acute myelogenous leukemia (AML) and possibly lymphomas in humans and animals [96-98]. As AA occurs 2-4 times more frequently in the Far East than in the West with unknown reasons, Dr. Young and his colleagues at the NHLBI have carried out the most comprehensive case-control study in Thailand in an attempt to identify possible etiologies of the disease. Consistent with previous reports, they found that exposure to benzene and other solvents and drugs significantly associated with AA [99]. Specifically, this study reported that benzene exposure for >4 days total increased the risk for developing AA by 3.5 fold [99]. While there was no association of AA with household pesticides, a significant association was noted with agricultural pesticides, such as organophosphates, DDT, and carbamates. Interestingly, farmers who were exposed to livestock, such as ducks and geese, were more likely to develop AA. Only borderline to no association was observed with the use of animal fertilizer or other chemical fertilizers, however. Drinking water from non-bottled sources, which may be contaminated with chemicals in the environment, was found to be strongly associated with the disease. There were too few chloramphenicol-exposed cases, which had been shown to be associated with AA in past studies (see below), to exclude its potential in the Thai's study.

A recent report has suggested that benzene exposure at levels even below the U.S. occupational standard of 1 part per million (ppm) can lead to significantly low white blood and platelet cell counts and decrease blood progenitor cell colony formations in cultures [94]. Several studies have suggested that the cytotoxic effect is caused by byproducts of the benzene metabolic pathway [100, 101]. The liver enzyme cytochrome P450 multifunctional oxygenase system first converts benzene into phenolic metabolites such as hydroquinone that is postulated to be a likely candidate responsible for hematotoxicity [102]. These metabolites can be further converted into highly reactive compounds, such as quinones and reactive oxygen species (ROS), by the peroxidase enzymes that reside in the bone marrow. Of these compounds, 1,4-benzoquinone and 1,2-benzoquinone are possibly most toxic in producing a reduction in hematogenesis [103]. These agents possibly cause direct toxic effects, including but possibly not limited to chromosomal damage (e.g., DNA strand breaks, telomere attrition), sister chromatid exchange, mitotic spindle damage, and inhibition of topoisomerase II enzyme. These and possibly other effects may lead to destruction of the bone-marrow stem cell compartment, resulting in marrow hypoplasia, which is the hallmark of AA [104-106].

Myeloperoxidase is present in the bone marrow and is likely the key enzyme to convert benzene metabolites to the ultra-toxic compounds [101]. Evidences showing that hydroquinone and other benzene-mediated metabolites alter the differentiation of marrow stem cells or induce cell death have been reported, although the exact mechanisms of

which are unknown [102, 107]. While it has been shown that benzene metabolites can covalently bind to DNA and protein of marrow cells in culture [108], its significance in vivo is less clear. ROS produced from the final conversion of benzene metabolites by the BM peroxidase can lead to oxidative stress in the marrow compartment. Oxidative stress is defined as a situation where the generation of ROS exceeds the ability of the cells to detoxify them and to repair structural or functional components of cells that are damaged by the free radicals. It has been well documented that irreversible damages to lipids or proteins in the cellular membranes by ROS can result in cellular apoptosis, necrosis or other forms of cell death [109]. For instance, increased levels of lipid peroxidation of the cell membranes as well as ROS and antioxidant levels have been noted in blood of children with AA and in mice exposed to benzene [110, 111]. Finally, it has been hypothesized that benzene or its metabolites may lead to the production of some yet unknown neo-antigens in the marrow that can mediate the autoimmune reaction that kills the marrow stem cells as is frequently observed in AA and other forms of BMFS.

Several animal models have been developed in order to understand the mechanism of benzene-induced bone marrow failure (for a review, see [112]). Exposure of mice and rats to benzene via inhalation or injection consistently produced hematopoietic damage [106, 113-118]. Benzene, or more accurately its metabolites, appears to alter expression of genes that regulate hematopoietic cell apoptosis, DNA repair, and cell cycle and growth controls [117, 119]. Benzene exposure may inhibit marrow stromal cells to produce sufficient amounts of cytokines necessary for normal hematopoietic cell growth and maintenance [114, 118]. It also causes a significant decline in total number of bone marrow cells with greatest net decreases in lymphoid and erythroid cells, suggesting that its main targets are the hematopoietic stem cells and progenitor stem cells [113].

Drugs-, radiation-, and virus-induced hematotoxicity

Chloramphenicol, an antibiotic originally isolated from *Streptomyces venezuela* [120], has a broad spectrum of antimicrobial activity and therapeutic efficacy. Its initial widespread usage was curtailed once it was found to be strongly associated with aplastic anemia and other marrow suppression syndromes [121]. It is still not clear how chloramphenicol can cause serious damage to the marrow. In the majority of the cases, anemia and leucopenia are reversed upon disruption of administration of the medication. Like benzene (see above), free radicals possibly released from the drug-induced injury to the mitochondria have been proposed to be the mechanism underlying hematotoxicity [122]. Chloramphenicol is less toxic than its derivative nitrosochloramphenicol that has been shown to inhibit DNA synthesis and cause irreversible inhibition of colony formation of marrow stem cells and cell death [123].

Busulfan, a drug used as a conditioning agent for allogeneic bone marrow transplantation, has also been shown to cause marrow failure when used inappropriately [124, 125]. Busulfan can cause significant defects in hematopoietic stem cell proliferation as has been clearly demonstrated in various animal studies [for a review, see [112]], although the exact mechanism underlying this effect is unknown. In addition to drugs, radiation has been shown to induce premature senescence of hematopoietic stem cells and progenitor cells as well as cellular apoptosis [126, 127].

Finally, it has been well documented that virus infections, such as that of parvovirus B19, can also lead to marrow failure syndromes [128, 129]. Mice infected with a strain of lymphocytic choriomeningitis virus (LCMV) exhibited pancytopenia and marked erythroid hyperplasia in the bone marrow [130, 131]. Infection by the cytomegalovirus in human or in mouse has also often been associated with transient neutropenia and thrombocytopenia [132, 133]. Again, the underlying mechanism for virally induced hematotoxicity is not well understood.

Environmental factors influencing human lung disease IPF

IPF is a progressive and fatal lung disease that is characterized by lung scarring and abnormal gas exchange effects [134]. In the U.S., there are about 89,000 known cases with approximately 34,000 new cases diagnosed each year [135]. Worldwide, the disease prevalence is 4 per 100,000 individuals at ages 18-34 but increases drastically to 227

per 100,000 in persons aged 75 and older and is most common in men who are smokers [135, 136]. Since IPF is an age-dependent disease, it has been speculated that a greater cumulative effect of environmental exposures in older adults than in children can play a role in the disease pathogenesis. Several studies have suggested that cigarette smoking is strongly associated with IPF. In one of the studies, half of the analyzed cases in families are smokers; and in other studies, older male smokers were especially more prone to the disease [137-139]. Indeed, cigarette smoking has been associated with a dose-dependent telomere shortening effect in human leukocyte [140]. More importantly, it was shown that carriers of telomerase mutations with a past history of smoking died on average 10 years sooner than non-smokers in families with IPF [56]. It is, therefore, possible that the disease is partly determined by both genotype and duration of tissue exposure to environmental toxicants.

Environmental factors influencing other human diseases with telomere shortening effect

During the past few years, experimental evidence has emerged to suggest that environmental factors may influence cellular proliferation and/or the rate of telomere attrition in an organ-specific manner. As mentioned above, cigarette smoking has been strongly associated with a dose-dependent shortening of telomere lengths in circulating leukocytes [140, 141]. Older patients (60-97 yrs old) with abnormally short telomeres have an 8-fold higher mortality due to infectious diseases compared to those with 'normal' (i.e., longer) telomeres [142]. Patients with atherosclerotic heart disease have significantly shorter telomere lengths than those of healthy age-matched controls [143-145]. Obesity, reduced bone marrow density, osteoporosis and cigarette smoking have been associated with shortened telomeres in women [141, 146]. Ulcerative colitis (UC) and other forms of chronic inflammatory bowel diseases (IBD) are associated with a high risk of carcinoma development in the gastrointestinal tract. Several studies have implicated chromosomal instability as a result of telomere attrition in IBD [147-149]. A greater degree of chromosomal abnormalities (e.g., losses and breakage-fusion bridges) due to shortened telomeres possibly as a result of oxidative stress was observed in biopsy samples of UC progressors than from non-progressor or healthy controls [147]. A recent study has shown that psychological stress is significantly associated with higher oxidative stress and shorter telomere length, which are known determinants of cellular senescence and longevity in peripheral blood of women [150]. It was shown that women with the highest levels of perceived stress due possibly to environmental stimuli have shorter telomeres by a decade of regular aging process as compared to those with low stress levels [150]. Again, this effect may likely influence earlier onset of some of the age-related diseases described above.

Possible mechanisms of telomere shortening effect caused by oxidative stress

The free radical theory of aging states that reactive oxygen species (ROS), a byproduct of metabolism, directly causes damage to the genome and other cellular components over the lifetime of an organism that can lead to its demise [151]. Increasing evidence has accumulated in support of this theory, based on antioxidant studies in worms and flies and caloric restriction studies in mice and other rodents (for a review, see [152]). The rates of telomere shortening in human fibroblasts have been estimated to be at around 10-20 bps per cellular population doubling [5]. However, higher rates of telomere attrition have been observed in cells that posses higher peroxide levels, indicating less effective antioxidant defense in these cells [153, 154]. Several studies have shown that telomeric DNAs are highly sensitive to damage caused by oxidative stress, mitochondrial dysfunction, alkylation, or ultraviolet irradiation [155-159]. While the exact mechanism of oxidative-induced telomere shortening is unclear, studies have indicated that DNA with G-rich sequences, such as telomeres, are more prone to oxidative damage as guanine is the most readily oxidized among the four bases due to its lower reduction potential [160, 161]. Under high oxidative stress conditions, telomeres can get shorten even without DNA replication due mainly to telomeric double-strand breaks or improperly assembled telomeric T-loop structure [159, 162]. By contrast, telomere shortening under mild oxidative stress conditions requires DNA replication [155, 163-165]. Indeed, cells are less proficient at repairing damage at telomeric DNA caused by

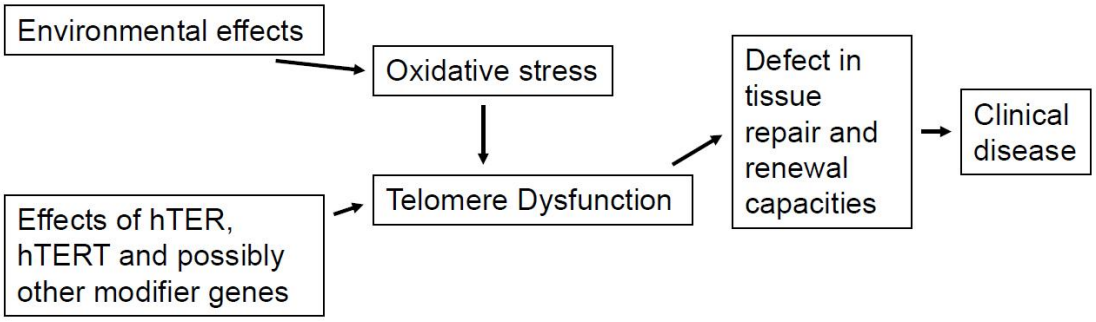

Figure 3: A proposed scheme of how genetic and environmental factors may work in concert to cause human diseases with telomere dysfunction. While disease-associated mutations in some components of telomerase (e.g., hTER, hTERT) can directly cause telomere dysfunction, the effect may also be caused or exacerbated by oxidative stress mediated through a cellular response to environmental factors (e.g., benzene or cigarette smoke) and/or by other yet unknown cellular gene products. Critically short or damaged telomeres can force cells into an arrested state (senescence) or to die (apoptosis) that can result in defect in tissue reserve, renewal and repair capacities. These effects collectively may lead to the clinical disease of AA and IPF.

oxidative or alkylative reaction than at other non-transcribed regions of the genome [158, 166]. Regardless of the mechanism, these studies have provided some evidence to show that one of the mechanisms of cellular senescence under stressful conditions is telomere shortening and that this effect is dependent on both external factors (e.g., environmentally induced ROS) and internal factors (e.g., genes involve in genome maintenance, antioxidant defense mechanism, and/or other cellular functions) (**Figure 3**).

Synthesis and perspectives

Despite recent advances in the understanding of the pathophysiology of human diseases acquired aplastic anemia and idiopathic pulmonary fibrosis, the possible causes of these diseases remain enigmatic. Numerous studies have documented environmental factors (e.g., benzene exposure in AA and cigarette smoking in IPF) to be highly associated with the diseases, the mechanisms of which are unknown. While the majority of AA and IPF cases are idiopathic in nature, a subgroup of patients in each disorder appears to exhibit a familial mode of inheritance. We and other researchers have recently shown that some of these patients are carriers of germline mutations in telomere-maintenance complexes. We have shown that cells isolated from some patients with the disease-associated mutations exhibited lower levels of telomerase enzymatic activity and markedly shorter telomere lengths than those of healthy age- and gender-matched individuals [42, 43, 53]. We propose that changes in telomere maintenance coupled with environmental and other genetic factors underlie the mechanism leading to AA and IPF. Further works to evaluate the functional consequences of both genetic and environmental factors influencing the pathogenesis of AA and IPF are required. Knowledge learned from these studies will likely lead to the development of novel therapeutic strategies that may benefit those who suffer from serious and fatal forms of human diseases with telomere dysfunction.

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